Symposium : Hematology/Oncology-Part II Indian J Pediatr 1998; 65 : 805-814

Cell Cycle Control and Cancer

Hans Peter Wagner

University of Bern, Bern, Switzerland

Abstract : This review consists of two parts. In the first part normal mechanisms regulating the progression of cells through the cell cycle are briefly reviewed. Besides mitogenic stimulation, cyclin kinase inhibition, the G1 restriction point and the prb pathway, accuracy of DNA replication and DNA repair, the G2 to M transition, apoptosis and the p 53 pathway, proteolytic, in particular ubiquitin - dependent mechanisms involved in the initiation of DNA synthesis in the separation of sister chromatids and in the telophase to GO/G1 transition, are discussed. In the second part oncogene and tumor suppressor gene products are briefly characterized. Aberrations of cell cycle control mechanisms associated with cancer are grouped as follows : deregulation of protooncogenes by translocations juxtaposing protooncogenes to immunoglobulin - or T cell receptor genes; translocations producing chimeric proteins unique to cancer cells; inversions and amplifications resulting in over expression of regulator genes; and deletions and mutations of tumor suppressor genes. It is emphasized that cancer is the result of a multistep process and that uncontrolled cell production and other alterations are, as a rule, late phenomena. **(Indian J Pediatr 1998; 65 : 805-814)**

***Key words** : Cell cycle; Regulator proteins; Checkpoints; Cancer; Chromosomal aberrations.*

Cancer research has contributed significantly to a better understanding of basic biological phenomena such as cell division, cell differentiation and apoptosis. By studying pathologic alterations important informations on the structure, function and regulation of normal cells have been obtained. In this review the focus will be on cell cycle control mechanisms and on control deficits associated with cancer.

Normal Cell Cycle Control Mechanisms

Cell replication is a complex, well controlled process[1,2]. In each phase of the cell cycle, cells that are not prepared to enter the next phase are arrested at checkpoints, synonymous to blocks controlled by surveillance mechanisms[2,3,4]. In this way high-fidelity replication of the approximately 3 billion base pairs during the S phase is achieved and precise segregation of the newly formed chromosomes is guaranteed.

Reprint requests : H.P. Wagner, M.D., Professor of Pediatrics, Schneiderstrasse 45, 3084 Wabern, Switzerland

Mitogenic stimulation

The initiation of replication depends on extracellular signals. Receptors at the cell surface bind ligands and activate intracellular signal transmission pathways to the nucleus. Other ligands may block receptors and preclude signal transmission. The nucleus of a cell has to receive a series of signals within a certain time, before replication is initiated. Under sustained mitogenic stimulation a cell prepares itself for replication[5,6]. No or insufficient preparation occurs, if the cell is dif-

ferentiated or if there is a lack of nutrients. Cells completely unprepared to divide but able to replicate are called G0 cells; cells partially prepared are in early G1.

Early G1 is characterized by the fact that extracellular mitogenic stimuli can turn on, probably by regulating the activity of nuclear transcription factors such as c-fos, c-jun, c-myc and others, the synthesis of cyclin dependent kinases (cdks). These kinases coordinate the transition of cells through the 4 phases of the cell cycle G1, S, G2 and M[2,4]. In order to be active the cdks have to complex with cyclins and the holoenzyme has to be activated by the cdk activating kinase CAK, a multi-subunit enzyme consisting of cyclin H and cdk7 (= MO 15)[7,8]. CAK plays an important role in the regulation of transcription. In mammalian cells CAK is part of TFIIH, a basal transcription factor containing helicase, ATPase and nucleotide-excision repair activities. TFIIH is able to phosphorylate the carboxy-terminal domain (CTD) of RNA polymerase II[9,10] Mitogens can only stimulate the synthesis of D-type cyclins. All other cyclins are synthesized at specific points during the cell cycle independent of extracellular stimuli.

Cyclin kinase inhibitors

The formation of active cyclin D - cdks can be blocked in many ways. Cdk inhibitors of the $p21^{WAF\,1/CIP1,}$ $p27^{KIP1}$ or $p57^{KIP2}$ or of the $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$ or $p19^{INK4d}$ type bind to the cdks and block either the holoenzyme formation with cyclin or the phosphorylation at threonin 172, by CAK[7]. INK4 inhibitors of cdk4 antagonize only cyclin D - $p21^{CIP1}$, $p27^{KIP1}$ and $p57^{KIP2}$ antagonize cyclin D-and cyclin E- or A-dependent kinases[11]. The $p21^{CIP1}$ is of particular interest since it binds also to the proliferating cell nuclear antigen (PCNA) which is a subunit of the DNA polymerase delta enzyme complex[12]

G1 restriction point

Active cyclin D-cdks phosphorylate the retinoblastoma tumor suppressor protein prb[13]. In the unphosphorylated state, the prb and the prb-related proteins p107 and p130 bind to transcription factors such as E2F and abl and arrest cells in G1. After phosphorylation these transcription factors are released and activate genes, the products of which are required for the transition from G1 to S, such as dihydrofolate reductase, DNA polymerase alpha, thymidine kinase and others[14]. With the phosphorylation of the prb the restriction-or-checkpoint in G1 is passed and from there on, cell replication proceeds without additional mitogenic stimulation.

Both extra-and intracellular stimulatory and inhibitory factors are therefore integrated before the cell replication machinery is irrevocably initiated. Once it is started, the cell either divides or dies.

Initiation of DNA synthesis

Towards the end of G1 the pre-replication complex (pre-RC) is assembled at DNA replicating origins[2,15]. This complex represents a DNA replication licensing system assuring that each DNA sequence is replicated only one time before chromosome segregation[16]. The pre-RC is composed of the origin recognition complex (ORC), an unstable protein called pcdc6, and the minichromosome maintenance proteins (mcms).

After phosphorylation of prb, ESF-DP heterodimers trigger the expression of cyc-

lin E and probably of cyclin A. The activation of cyclin E-and cyclin A-cdk2 initiates DNA synthesis and inhibits any de novo formation of pre-RC[17]. Another cyclin-cdk independent kinase, cdc7p-dbf4p (DDK) also interacts with components of the pre-RC[15].

After initiation of DNA synthesis, cyclin E is inactivated by ubiquitin-dependent proteolysis (see below), and the DP components of heterodimeric ESFs are phophorylated by cyclin A-cdk2, precluding DNA binding of E2Fs[11]. Cyclin A synthesis proceeds throughout S and reaches peak values in G2. In early S. cyclin A is associated with cdk2, later with cdc2.

Accuracy of DNA replication and DNA repair

The accuracy of DNA replication is checked by proofreading functions coupled to the DNA polymerization activities. TFIIH (see above) plays an important role[18].

The gene ATM (= ataxia telangiectasia muted gene) appears to be involved in sensing DNA damage induced by irradiation and other agents. It activates the TP53 gene on 17p13[19-21]. p53 acts as a transcription factor[22] p53 is phoshorylated specifically by cyclin A - cdk2 and cyclin B-cdc2, but not by cyclin D1-cdk4 or cyclin E-cdk2. Phosphorylation dramatically stimulates the binding of p53 to its targets in the $p21^{WAF1/CIP1}$ and GADD45 genes[10]. p53 induces a block at the G1-S checkpoint and slows the progression of cells in S and G2[11]. p53 also promotes apoptosis[23,24].

Incomplete replication of telomeres is counteracted by telomerase, an enzyme that may be lost during differentiation. Telomeres, GC-rich sequences at the ends of each chromosome, are not completely copied during DNA synthesis, because DNA polymerases require an RNA primer to start and because they proceed only in the 5' to 3' direction[25]. Without telomerase, the potential of a cell to divide is limited (cell aging)[26,27].

G2 to M transition

The transition from G2 to M is also orchestrated by cyclin-dependent kinase activities, mainly cyclin B-cdc2. Cyclin B is first synthesized in late S and peaks in late G2 or early M[11]. Cyclin B-cdc2 is activated by phosphorylation at threonine 161. Many other factors, e.g. tyrosine kinases and phosphatases, control cyclin B-cdc2 activity. During mitosis active cyclin B-cdc2 phosphorylates various substrates, e.g. B-type nuclear envelope lamins, at mitosis-specific sites in the N-terminal domain. Phosphorylation of these sites induces lamina depolymerization and nuclear membrane dissolution[28]. Cyclin B-cdc2 is also involved in the repression of RNA polymerase III[10]

Ubiquitination, sister chromatid separation and telophase to G0/G1 transition

Mitotic cyclins control the condensation of chromosomes, the spindle formation and the alignment and attachment of all pairs of sister chromatids to the mitotic spindle. For the latter process surveillance mechanisms exist detecting sister kinetochores which have not been properly aligned[4,17]. These mechanisms block the activation of the anaphase-promoting complex (APC) or cytosome, a large multisubunit complex essential for chromosome splitting and destruction of M-phase cyclins[2,17].

APC and other proteolytic cell cycle enzymes target substrates for 26S proteasome degradation by ubiquitination, i.e. assembling an ubiquitin chain on the substrate. Proteolytic degradation of the PDS1 and CUT2 proteins is essential for sister chromatid separation, and ubiquitinisation of M-phase cyclins for deblocking both the telophase to G0/G1 transition and the pre-RC assembly[17].

Cell cycle exits

Cells exit from the cell cycle either by differentiation or by necrotic or apoptotic cell death. Differentiation is probably restricted to cells in G0/early G1; death can occur in any phase of the cell cycle. Apoptosis is an energy-dependent programmed cell death in response to certain stimuli, associated with characteristic morphologic and biochemical features. Apoptosis can be induced by irradiation, chemotherapy, viral infections, cytotoxic lymphocyte killing, and growth factor or hormone withdrawal[29-32]. Some genes active in the cell cycle appear to influence apoptosis[29-32] e.g. c-myc, p53 and prb[24]. Apoptosis is another mechanism regulating cell production[33].

Aberrations of Cell Cycle Control Mechanisms Associated with Cancer

In solid tumors very complex chromosomal anomalies are found, even if primary tumours are examined : hyperdiploidy to near tetraploidy, structural abnormalities concerning the centromeric regions, amplifications, loss of chromosomes, deletions and mutations. Mutations in genes controlling checkpoints can relieve arrest signals and result in continued, uncontrolled progression through the cell cycle. A dysregulation of cell cycle control is virtually pathognomic of all cancer cells.

Oncogenes and Tumor Suppressor Genes

Oncogenes and tumor suppressor genes have been identified by studying cancer-causing viruses, by assessing cancer genes in tissue cultures, by analyzing genes at sites of chromosomal aberration in neoplastic cells and by isolating genes for cancer-predisposing familial syndromes.

Oncogene products or products of inappropriate or mutated protooncogene expression increase cell production. They function as growth factors [e.g. as platelet-derived growth factor (PDGF)], growth factor receptors [e.g. for epidermal growth factor (EGFR)], proteins involved in signal transduction (e.g. upregulating RAS-related signaling pathways), transcription factors (e.g. c-myc), or antiapoptosis proteins (e.g. BCL-2). Overexpression of oncogenes can be the result of gene amplification, translocations or inversions. If germ line cells are affected, inheritance is dominant[34].

Tumor suppressor gene products, e.g. prb, p53, p16 and apoptosis promoting proteins such as bax, reduce or block cell production. Tumor suppressor genes can be lost or mutated or their products can be inactivated, e.g. by viral oncoproteins of adenovirus (E1A), SV40 (large T antigen) and human papilloma-viruses (E6 and E7). If germ line cells are affected, inheritance is recessive (although some mutants may be dominant)[35].

Chromosomal Aberrations in Cancer and Cell Cycle Control

How frequently structural rearrangements occur under normal circumstances is

unknown. Inherent genetic instability or fragile sites may mediate rearrangements. Chromosomal rearrangements are present in virtually all malignant cells. A number of specific cytogenetic abnormalities have been recognized that are closely or even uniquely associated with clinically distinct subsets of leukemias or solid tumors.

Translocations Causing Deregulation of Gene Expression

Chromosomal translocations can lead to a deregulation of gene expression, either an aberrant expression in a tissue that does not normally express the gene, or an overexpression. Examples are the translocations involving immunglobulin (Ig) or T-cell receptor (TCR) genes[36].

In Burkitt's lymphoma and B-cell ALL the MYC gene at 8q24 is juxtaposed to an Ig gene, either the heavy chain gene at 14q32, the κ light chain gene at 2p12, or the λ light chain gene at 22q11, by an 8; 14, an 2; 8 or an 8; 22 translocation, respectively. This juxtaposition results in an abnormal regulation of MYC expression. The c-myc protein localizes to the nucleus and interacts, by heterodimerization, with MAX and other regulators of the myc translational system. Transcription from c-myc target genes is activated by MYC - MAX and is repressed by MAD-MAX or MXI-MAX heterodimers[37-40]. A c-myc activation stimulates cell proliferation in the absence of growth factors. In cells under nutrient deprivation, c-myc induces apoptosis. In quiescent cells c-myc expression results in activation of cyclin E and A, while cyclin D may be repressed. In signaling pathways c-myc is an essential component, mediating transformation by oncoproteins such as bcr-abl, CSF-1 receptor tyrosine kinase and RAS.

In follicular lymphoma the BCL2 gene on 18q21 is juxtaposed by translocation to the lg heavy chain gene at 14q32. The resulting chimeric protein blocks apoptosis[36].

In B lineage mantle cell lymphomas t (11; 14) (q13; q32) puts the lg heavy chain inducer on 14q32 into the cyclin D1 locus leaving the D 1 coding sequences uninterrupted[11]. While normal B lymphocytes express only cyclins D2 and D3, all lymphoma cells with a t(11 ; 14) ectopically synthesize cyclin D1 (cyclin D1 overexpression in other solid tumors : see below).

In T-cell disorders, TCR β-and δ-chain; β; or γ-chain genes at 14q11; 7q34- 35 and 7p15, respectively, are juxtaposed by translocations to genes encoding transcription factors, such as TAL1, LYL1 and TAL2[36,41].

Translocations Producing New Fusion (chimeric) Proteins

Translocations can produce novel fusion proteins resulting from the juxtaposition of coding sequences normally located on different chromosomes. Fusion proteins are only observed in the cancer cells and may therefore be important in diagnosis.

Leukemias

Examples for fusion proteins are the bcr/abl protein [CML, t (9; 22)], the AML 1/ETO protein [AML type M2 (M 4), t (9; 21)], the PML/RARA protein [AML type M 3, t (15, 17)], the E 2 A/PBX 1 protein [pre-B ALL, t (1; 19)] and a number of fusion proteins resulting from translocations involving the MLL gene at 11 q 23 [e.g. AML type M 5, t (9; 11)] infant ALL, t

(4; 11)][36]. The p $210^{bcr\text{-}abl}$ is located on the cytoplasmic surface of the cell membrane and transmits growth-regulatory signals from the cell surface receptors via the ras signal transduction pathway to the nucleus[42]. Tyrosine kinase inhibitors inhibiting the growth of cells expressing bcr-abl or related fusion proteins have been described[43]. The core binding factor α (CBFα), the normal product of AML1, complexes with the core binding factor β (CBFβ) to form a transcription factor regulating the expression of genes critical to myeloid cell growth, differentiation and function such as IL3, GM-CSF, MPO and CSF 1 receptor. The AML1/ETO protein binds to the regulatory regions of these genes but cannot activate their expression[44,45].

The PML/RARA protein is released on exposure to all-trans-retinoic acid and activates retinoic acid responsive gene transcription inducing differentiation of APL cells[35,44,46].

E2A, the product of a gene on 19 q 13, functions as a transcription factor. The gene on 1q21 is a homeobox gene. In the fusion protein the DNA-binding protein of E 2 A is replaced by that of the PBX 1 gene which may alter the target genes of the chimeric transcription factor[47].

The MLL gene encodes a transcription factor with a zinc finger domain, a DNA binding AT-hook, a DNA methyltransferase domain and transcription activation and repression domains. In all fusion proteins with the MLL protein, the latter has lost the activation domain but retains the DNA binding and repression domains[36,44,48,49].

Solid tumors

In a large proportion of rhabdomyosarcomas a t (2; 13) (q35; q14) creating a fusion transcription factor involving the PAX 3 homeobox gene on 2 q 35 and the FKHR (forkhead domain) on 13 q 14, was observed. A variant, t (1; 13) (p36; q14), was also described[36,50].

In Ewing's sarcoma, peripheral neuroepithelioma, Askin's tumor, esthesioneuroblastoma and a few medulloblastomas a t (11; 22) (q24; q12) is observed. The translocation results in a chimeric transcription factor containing the transactivation domain of the EWS gene (22q12) and the DNA binding of the EL11 gene (11q24)[35,51,52].

Inversions and Amplifications Leading to Overexpression of Cell Cycle Regulators or Protooncogenes

In parathyroid adenomas an i(11)(p15;q13) links cyclin D1 to parathyroid hormone[53]. Cyclin D1 (formerly PRAD1) encoded on 11q13 (formerly CCND1) and CDK4 encoded on 12q13 (together with the p53 antagonist MDM2) are overexpressed in sarcomas and gliomas[54,55]. Amplification of the D1 locus was observed in almost half of head and neck squamous cell carcinomas, in about a third of esophageal carcinomas and in bladder cancers, primary breast cancers, small-cell lung cancers and hepatocellular carcinomas[56]. Aberrant overexpression of cyclin D1 was also seen in sarcomas, colorectal tumors and melanomas even though D1 gene amplification frequencies were low[56]. In mice overexpression of D1 in mammary epithelial cells leads to hyperproliferation and tumor formation, while animals nullizygous for D1 show severe defects in mammary lobuloalveolar development during pregnancy[57,58].

In neuroblastomas predominantly deletions or rearrangements of the terminal portion of 1p are found. Loss of heterozygosity for 1p36.2-3. was preseent in 26% of primary neuroblastomas[59]. In up to a third of the cases amplification of the N-MYC oncogene is observed[60].

Deletions and Mutations of Tumor Suppressor Genes

Retinoblastoma is the hallmark of a tumor due to the loss of a tumor suppressor gene, the RB1 gene on 13q14. Both alleles have to be deleted, before retina cells become neoplastic. Inactivation of the RB gene is also seen in osteosarcoma, carcinoid tumors, small-cell lung cancers and invasive bladder cancers[61]. As indicated previously, the RB product, prb, can also be inactivated by viral oncoproteins, e.g. human papillomavirus protein E7[61,62].

Inactivation of the INK4a locus (on 9p21) by homozygous deletion, methylation of the promotor or point mutation occurs in approximately 50-60% of gliomas, head and neck carcinomas, mesotheliomas and T-All, in about 25-50% of bladder and pancreas cancer and melanoma as well as in oesophagus, gastric, non-small-cell lung, renal and prostate cancers[56,63]. Mutations of the INK4a (=CDKN2 or MTS1) gene on 9p21 resulting in production of inactive $p16^{INK4a}$, are associated with familial melanoma and are seen in a high proportion of biliary tract and esophageal carcinomas[64,65]. p16 is frequently detected in premalignant lesions. It was suggested, therefore, that p16 inactivation represented an early event in tumor development[66]. p16 is also inactivated by human papillomavirus protein E6[62]. Loss of $p16^{INK4a}$ might mimic cyclin D1 or CDK4 overexpression, both leading to hyperphosphorylation and inactivation of prb.

The TP53 gene on 17p13 is the most frequently mutated gene in cancer[67]. Loss of TP53 gene alleles, or mutations associated with nuclear accumulation of missense p53, are characteristically seen in more advanced cancers. In patients with colorectal cancer, up to 80% were found to have deletions of 17p13 and the rest had mutations. In breast cancer, up to 50% had losses or mutations of the p17p13 gene and in many other cancers such as lung and liver, head and neck, pancreas, oesophageal, ovarian, endometrial and cervical cancers as well as lymphomas, sarcomas and brain tumors nuclear accumulation of missense p53 was found by immunostaining. By examining the spectrum of p53 mutations in a series of tumors, environmental or endogenous factors involved in tumorigenesis might be recognized[68].

Cancer is the result of a multistep process. If, e.g., chromosomal translocations result in a loss of checkpoint control and uncontrolled proliferation, the increased cell production can still be compensated by increased apoptotic death. Only if a second mutation blocks apoptosis, decompensation and accumulation of malignant cells occurs. As genetic instability increases due to diminished cell cycle control, the malignant cells may acquire additional characteristics such as invasiveness and decreased drug sensitivity.

Conclusions

This review demonstrates that human cancers are often associated with inherited or acquired chromosomal aberrations leading to overexpression or repression of genes that play an important role in cell cy-

cle replication, differentiation and/or apoptosis. It is also evident, that important decisions, e.g. the triggering of cell division or of apoptosis, or the induction of cell differentiation, are subject to very complex steering mechanisms, that take into account a multitude of extrinsic and intrinsic factors. It is not astonishing, therefore, that cancer does not result from one, but from at least two or more genetic defects or events. There is good evidence to suggest that cancer develops stepwise, and that uncontrolled cell proliferation as well as invasiveness and metastatic spreading or the development of drug resistance, are, as a rule, late phenomena of a long lasting process. It is therefore not surprising, that older individuals are more often affected than younger ones. By extending our understanding of basic biological phenomena, molecular biology and genetics are poviding bases for more rational diagnostic and therapeutic approaches, by offering alternatives to actual strategies and by defining promising targets for new therapeutic efforts.

REFERENCES

1. Murray AW, Kirschner MW. Dominoes and clocks : the union of two views of the cell cycle. *Science* 1989; 247 : 614-621.
2. Nasmyth K. Viewpoint. putting the cell cycle in order. *Science* 1996; 274 : 1643-1645.
3. Hartwell LH, Weinert TA. Checkpoints : Controls that ensure the order of cell cycle events. *Science* 1989; 246 : 629-634.
4. Elledge SJ. Cell cycle checkpoints : preventing an identity crisis. *Science* 1996; 274 : 1664-1672.
5. Pardee AB. G1 events and regulation of cell proliferation. *Science* 1989; 246 : 603-608.
6. Aronson S. Growth factors and cancer. *Science* 1991; 254 : 1146-1153.
7. Fisher RP, Morgan DO. A novel cyclin associates with MO15/cdk7 to form a cdk-activating kinase. *Cell* 1994; 78 : 713-724.
8. Morgan DO. Principles of cdk regulation. *Nature* 1995; 374 : 374 : 131-134.
9. Nigg EA. Cyclin dependent kinase 7 : at the crossroads of transcription, DNA repair and cell cycle control? *Curr Opin Cell Biol* 1996; 8 : 312-317.
10. Dynlacht BD. Regulation of transcription by proteins that control the cell cycle. *Nature* 1997; 389 : 149-152.
11. Sherr CJ. Cancer cell cycle. *Science* 1996; 274 : 1672-1677.
12. Jacks T, Weinberg RA. The expanding role of cell cycle regulators. *Science* 1998; 280 : 1035-10
13. Weinberg RA. The retinoblastoma protein and cell cycle control. *Cell* 1995; 81 : 323-330.
14. Dyson N. pRB, p107. and the regulation of the E2F transcription factor. *J Cell Sci (suppl)* 1994; 18 : 81-87.
15. Stillmann B. Cell cycle control of DNA replication. *Science* 1996; 274 : 1659-1664.
16. Chong JPJ, Mahbubani HM, Khoo C-Y, Blow JJ. Purification of an MCM-containing complex as a component of the DNA replication licensing system. *Nature* 1995; 375 : 418-421.
17. King RW, Deshaies RJ, Peters J-M, Kirschner MW. How proteolysis drives the cell cycle. *Science* 1996; 274 : 1652-1659.
18. Sancar A. Mechanisms of DNA excision repair. *Science* 1994; 266 : 1954-1956.
19. Brown L, McCarthy N. A sense-abl response ? *Nature* 1997; 387 : 450-451.
20. Baskaran R, Wood LD, Whitaker LL *et al.* Ataxia telangiectasia mutant protein activates c-able tyrosine kinase in response to ionizing radiation. *Nature* 1997; 387 : 516-519.
21. Vogelstein B, Kinzler KW. p53 function and dysfunction. *Cell* 1992; 70 : 523-526.
22. Prives C, Manfredi JJ. The p53 tumor suppressor protein. *Genes Dev* 1993; 7 : 529-534.

23. Wyllie A. Apoptosis. Clues in the p53 murder mystery. *Nature* 1997; 389 : 237-238.
24. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. *Nature* 1997; 389 : H 300-305.
25. Greider CW, Blackburn EH. Telomeres, telomerase and cancer. *Sci Amer* 1996; 274 (2) : 80-85.
26. De Lange T. Telomeres and senescence : ending the debate. *Science* 1998; 279 : 334-335.
27. Bodnar AG, Quelette M, Frolkis M *et al.* Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998; 279 : 349-352.
28. Peter M, Nakagawa J, Doree M, Labbé JC, Nigg EA. In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. *Cell* 1990; 61 : 591-602.
29. Duke RC, Ojcius DM, Young JD-E. Cell suicide in health and disease. *Sci Amer* 1996; 274 (6) : 48-55.
30. Nagata S, Goldstein P. The fas death factor. *Science* 1995; 267 : 1449-1456.
31. Chinnaiyan AM, Chaudhary D, O'Rourke K, Koonin EV, Dixit VM. Role of CED-4 in the activation of CED-3. *Nature* 1997; 388 : 728-729.
32. Irmler M, Thome M, Hahne M *et al.* Inhibition of death receptor signals by cellular FLIP. *Nature* 1997; 388 : 190-195.
33. Reed JC. Double identity for proteins of the Bcl-2 family. *Nature* 1997; 387 : 773-776.
34. Krontiris TG. Oncogenes. *N Engl J Med* 1995; 333 : 303-306.
35. Weinberg RA. Tumor suppressor genes. *Science* 1991; 254 : 1138-1146.
36. Rabbits TH. Chromosomal translocations in humna cancer. *Nature* 1994; 372 : 143-149.
37. Gu W, Cechova K, Tassi V, Della-Favera R. Opposite regulation of gene transcription and cell proliferation by c-myc and Max. *Proc Natl Acad Sci* 1993; 90 : 2935-2939.
38. Ayer D, Kretzner L, Eisenmann R. Mad, a heterodimeric partner for Max that antagonizes Myc transcriptional activity. *Cell* 1993; 72 : 211-222.
39. Zervos A, Gyuris J, Brent R. Mxi, a protein that specifically interacts with Max to bind Myc-Max recognition sites. *Cell* 1993; 72 : 223-232.
40. Amati B, Brooks M, Levy N *et al.* Oncogenic activity of the c-myc protein requires dimerization with Max. *Cell* 1993; 72 : 233-245
41. Uckun FM, Sensel MG, Sun L *et al.* Biology and treatment of childhood T-lineage acute lymphoblastic leukemia. *Blood* 1998; 91 : 735-746.
42. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-able oncogene products. *Science* 1990; 247 : 1079-1082.
43. Carroll M, Ohno-Jones S, Tamura S *et al.* CGP 57148, a tyrosine kinase inhibitor inhibits the growth of cells expressing BCR-ABL, TEL-ABL, and TEL-PDGFR fusion proteins. *Blood* 1997; 90 : 4947-4952.
44. Tenen DG, Hromas R, Licht JD, Zhang D-E Transcription factors, normal myeloid development and leukemia. *Blood* 1997; 90 : 489-519.
45. Okuda T, Cai Z, Yang S *et al.* Expression of a knocked-in AML1-ETO leukemia gene inhibits the establishment of normal finitive hematopoiesis and directly generates dysplastic hematopoietic progenitors. *Blood* 1998; 91 : 3134-3143.
46. Benedetti L, Levin AA, Scicchitano BM *et al.* Characterization of the retinoid binding properties of the major fusion products present in acute promyelocytic leukemia cells. *Blood* 1997; 90 : 1175-1185.
47. Hunger SP. Chromosomal translocations involving the E2A gene in acute lymphoblastic leukemia : clinical features and molecular pathogenesis. *Blood* 1996; 87 : 1211-1224.
48. Poirel H, Rack K, Delabesse E *et al.* Incidence and characterization of MLL gene

(11q23) rearrangements in acute myeloid leukemia M1 and M5. *Blood* 1996; 87 : 2496-2505.

49. Behm FG, Raimondi SC, Frestedt JL *et al.* Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. *Blood* 1996; 87 : 2870-2877.
50. Kelly KM, Wamer RB, Sorensen PHB, Xiong QB, Barr FG. Common and variant gene fusions predict distinct clinical phenotypes in rhabdomyosarcoma. *J Clin Oncol* 1997; 15 : 1831-1836.
51. Delattre O, Zucman J, Melot T *et al* The Ewing family of tumors - a subgroup of small-round-cell tumors defined by specific chimeric transcripts. *N Engl J Med* 1994; 331 : 294-299.
52. Zoubek A, Dockhorn-Dwornizak B, Delattre O *et al.* Does expression of different EWS chimeric transcripts define clinically distinct risk gorups of Ewing tumor patients? *J Clin Oncol* 1996; 14 : 1245-1251.
53. Motokura T, Bloom T, Kim HG *et al.* A novel cyclin encoded by a bcl1-linked candidate oncogene. *Nature* 1991; 350 : 512-515.
54. Khatib ZA, Matsushime H, Valentine M, *et al.* Coamplification of the CDK4 gene with MDM2 and GL1 in human sarcomas. *Cancer Res* 1993; 53 : 5535-5541.
55. He J, Allen JR, Collins VP *et al.* CDK4 amplification is an alternative mechanism to p16 gene homozygous deletion in glioma cell lines. *Cancer Res* 1994; 54 : 5804-5807.
56. Hall M, Peters G. Genetic alterations of cyclins, cyclin-dependent kinases and cdk inhibitors in human cancer. *Adv Cancer Res* 1996; 68 : 67-108.
57. Wang TC, Cardiff RD, Zuckerberg L Lees E, Arnold A, Schmidt EV. Mammary hyperplasia and carcinoma in MMTY-cyclin D1 transgenic mice. *Nature* 1994; 369 : 669-671.
58. Sicinski P, Donaker JL, Parker SB *et al.* Cyclin D1 provides a link between development and oncogenes in the retina and breast. *Cell* 1995; 82 : 621-630.
59. White PS, Maris JM, Beltinger C *et al.* A region of consistent deletion in neuroblastoma maps within 1p36.2-2.3 *Proc Natl Acad Sci USA* 1995; 92 : 5520-5524.
60. Tonini GP, Boni L Pession A. *et al.* MYCN oncogene amplification in neuroblastoma is associated with worse prognosis, except in stage 4s; the Italian experience with 275 children. *J Clin Oncol* 1997; 15 : 85-93.
61. Sellers WR, Kaelin Jr WG. Role of the retinoblastoma protein in the pathogenesis of human cancer. *J Clin Oncol* 1997; 15 : 3301-3312.
62. Alani RM, Munger K. Human papillomavirus and associated malignancies. *J Clin Oncl* 1998; 16 : 330-337.
63. Liggett JR WH, Sidransky D. Role of the p16 tumor suppressor gene in cancer. *J Clin Oncol* 1998; 16 : 1197-1206.
64. Hussussian CJ, Struewing JP, Goldstein AM *et al.* Germline p16 mutations in familial melanoma. *Nature Genet* 1994; 8 : 15-21.
65. Goldstein AM, Fraser MC, Struewing JP *et al.* Increased risk of pancreatic cancer in melanoma-prone kindreds with $p16^{INK4a}$ mutations. *N Engl J Med* 1994; 333 : 970-974.
66. Reznikoff CA, Yeager TR, Belair CD *et al.* Elevated p16 at senescence and loss of p16 at immortalization in human papillomavirus 16 E6, but not E7, transformed human uroepithelial cells. *Cancer Res* 1996; 56 : 2886-2890.
67. Hollstein M, Sidransky D, Vogelstein B, Harris C. p53 mutations in human cancer. *Science* 1991; 253 : 49-53.
68. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. *N Engl J Med* 1993; 1318-1327.